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BOOK OF ABSTRACTS

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Detection of RIF and INH resistance of *Mycobacterium tuberculosis* using TaqMan-based real-time PCR assays: A preliminary study

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Introduction and Objectives: The timely and accurate initiation of first-line anti-tuberculosis (TB) drugs is demanded, as 90% of rifampin (RIF) resistant TB (RR-TB) are also resistant to isoniazid (INH). The current diagnosis of RR-TB is mainly targeting the mutations within the 81 bp hotspot region of the *rpoB* gene. In our study, we describe single-plex and duplex TaqMan-based real-time PCR (RT-PCR) assays using self-designed primers and probes (FAM/HEX) to co-identify RR-TB and/or isoniazid-resistant TB (Hr-TB) including mutations outside the hotspot region.

Methods: The genes/regions targeted were *rpoB* (four primer-probe pairs: P1-(426-432), P2-(432-439), P3-(439-446) and P4-(446-452)), the inhA promoter region (upstream fabG1) and katG. All assays were performed using Rotor Gene-Q duplex RT-PCR instrument. The *IS6110* gene was included to differentiate *Mycobacterium tuberculosis* (MTB) from non-tuberculous mycobacteria. Firstly, all primers and probes were optimized using H37Rv. The clinical isolates with mutations previously identified in the laboratory via whole-genome sequencing were used for validation (NCBI accession numbers: SAMN18650581, SAMN18650579, and SAMN18650578). Each PCR master mixture contained 25 µl of, 5X PCR buffer 5 µl, dNTP (0.4 mM) 2.5 µl, MgCl2 (25 mM) 2 µl, HotStar Taq DNA polymerase (Qiagen) (5U/ µl) 0.2 µl, final concentrations of primers and probes were 200 nM and 250 nM, respectively and 75ng of the template. The cycling program was as follows: Initial denaturation at 95 °C for 10 mins, 45 cycles of denaturation at 95 °C for 30 secs, combined annealing, and extension at 60 °C for 60 secs. Milli-Q water was used as negative control.

Results: The results were 100% concordance with both single-plex and duplex TaqMan-based RT-PCR assays. Ct values from 15–40 were considered positive (no mutation), while Ct values equal to zero or equal to or greater than 40 were considered negative (mutation present=drug resistant). This was further tested using the DNA of 11 phenotypically as well as genotypically confirmed drug-resistant and 10 drug sensitive MTB isolates.

Conclusions: RT-PCR assay is a simple, rapid, and accurate molecular diagnostic method to detect RR-TB and Hr-TB. However, further validation is needed on a large scale to improve the assays.

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Keywords: TaqMan-based RT-PCR, drug-resistance, isoniazid, rifampin